Occurrence of Mycobacterium Other than Mycobacterium tuberculosis in the Oral Cavity and in Sputum

CRETYL C. MILLS
William Pepper Laboratory, Microbiology Division, Department of Pathology, School of Medicine, University of Pennsylvania, Philadelphia, Pennsylvania 19104

Received for publication 17 June 1972

Mycobacteria were cultured from 9% of 424 paired mouthwash-induced sputum specimens. The majority of the organisms were not Mycobacterium tuberculosis. Sputum cultures contributed 59% of these isolations. M. intracellularare was the species most frequently isolated. The non-M. tuberculosis mycobacteria may constitute part of the oral flora of the general population and are not more prevalent in hospitalized patients. Approximately one-third more isolations were made from the patients furnishing two or three pairs of specimens as compared to those patients providing one pair of specimens each. From the paired specimens of 4 of 113 patients, two species of Mycobacterium were isolated. M. intracellularare was isolated from two of three samples of tap water and from the fingers of 1 of 52 patients.

For at least 5 years cultures of clinical materials in the mycobacteriology laboratory of the Hospital of the University of Pennsylvania have yielded mycobacteria other than Mycobacterium tuberculosis in the ratio of 3:2 over M. tuberculosis. This study of mouthwash specimens was undertaken to determine if the oral cavity contributes these large numbers of clinically insignificant non-M. tuberculosis mycobacteria to sputum during sputum collection. Patients referred to the Induced Sputum Laboratory provided paired mouthwash-sputum specimens. Since this group of patients contained individuals from the Outpatient Clinic, it afforded an opportunity to determine if the non-M. tuberculosis organisms were present in the oral flora of the general population or if they were more prevalent in hospital patients.

MATERIALS AND METHODS

Mouthwash solution. Todd-Hewitt Broth (Difco), 20 ml sterilized in 50-ml screw-capped vials, was used as the mouth rinse to recover mycobacteria.

Sputum induction. A warmed aqueous solution consisting of 10% NaCl and 15% propylene glycol, nebulized by Mistogen Equipment Co. apparatus, was conducted into the patient's mouth by tubing, terminating in a disposable mouthpiece.

Specimen collection. Each specimen was collected in a sterile sputum collection kit (Falcon 9002) which minimized the chances of contamination during handling.

Mycobacterial culture media. Lowenstein-Jensen slants (Difco Lowenstein medium 1017) and direct cord reading agar (DCRA) plates were used. The DCRA plates were prepared by the method of Lorian (5) and consisted of 7H10 agar (Difco Middlebrook agar 0624) to which were added 0.5% Triton (Ruger Chemical Co., Irvington on Hudson, N.Y.) and oleic acid-albumin-dextrose-catalase enrichment (Difco Middlebrook OADC enrichment 0722).

Specimen decontaminant. To liquefy mucus and kill extraneous bacteria, to each specimen was added an approximately equal amount of a mixture of 9 parts of 2.2% NaOH solution and 1 part of Sputolysoin (6) (Calbiochem trade name for buffered dithiothreitol).

Mouthwash collection. Each patient first took directly into his mouth 20 ml of Todd-Hewitt broth. After use as a mouth rinse without gargling, the fluid was expectorated into a collection kit and labeled "mouthwash."

Induced sputum collection. After rinsing the mouth, the patient then produced induced sputum by repeatedly inhaling the warmed aerosol. The sputum was collected in another collection kit, labeled "induced sputum."

 Finger washings. After the studies with sputa and mouthwashes were completed, 52 patients coming to the Induced Sputum Laboratory rinsed their fingers as the 20 ml of Todd-Hewitt broth was poured from the vial onto their fingers and collected in a sputum collection kit. Processing of these
rinings was similar to that of the other specimens.

**Tap water contents.** During the course of these studies, two 7- and 20-liter samples of water were collected from a cold-water faucet at the nurses’ station and an 11.8-liter sample was collected from a water faucet in one of the patient’s rooms in the Infectious Disease Unit. The samples were collected in sterile flasks and filtered through 0.45-μm pore membrane filters (Millipore Corp.), 35-mm D with a maximum suction of about 27 psi. The water was filtered until the filters became clogged with the fine particulate material in the water, hence the varying volumes of the samples. Material on the surface of the filters was transferred directly by inverting the filters onto the surface of 7H10 agar, and the plates were incubated as for other specimens.

**Specimen processing.** Each pair of mouthwash-sputum specimens was labeled with the patient’s name and other pertinent information, sent to the laboratory by messenger, and processed by the following method which was standard for all mycobacteriological specimens.

This method was a modification of the Lorian technique (5). Vortex mixing was used to achieve visible liquefaction followed by an additional 30 sec of mixing. Decontamination took place during the ensuing 25 min at room temperature. Sterile water added to the 50-ml mark on the tube neutralized the decontaminant before centrifugation at 3,700 rev/min for 30 min. After the supernatant fluid was decanted, the sediment was mixed briefly, and a smear was prepared, heat-fixed on an electric warmer at 65°C for 2 hr, stained by the Ziehl-Neelsen technique, and examined microscopically according to standards recommended by Vestal (7), Center for Disease Control.

To the remaining sediment was added 1 ml of sterile 0.5% bovine albumin (Difco 0503) as a buffer. After brief mixing, samples of 0.1 ml were used to inoculate two Lowenstein-Jensen slants and a DCRA plate. The plates were inoculated by placing 10 drops directly on the agar surface at equidistant points and allowing 2 to 3 hr for liquid absorption before plate inversion. All cultures were incubated at 36°C with 5% added CO2. Slants, their caps loose during the first week, were examined weekly for 6 weeks at ×2 magnification. Plates, incubated in polyethylene bags, were examined for the appearance of cored (pathogenic) microcolonies at 12 and 21 days by using ×50 magnification. Any bacterial growth was stained, and that containing acid-fast bacilli was subjected to standard tests for species identification as recommended by Vestal (7). All procedures for decontamination and inoculation, except centrifugation, were performed within a laminar airflow Biohazard hood. Water for stain and cultures was membrane filtered (Millipore Corp.) before sterilization, and all glassware was cleaned with acid-dichromate before sterilization.

**Experimental specimens.** During 8 months, 20 June 1969 to 5 February 1970, 113 induced-sputum laboratory patients submitted 212 pairs of mouthwash-sputum specimens; 48 patients each provided single pairs of specimens; 65 patients provided two or more pairs each, and of these six submitted four pairs, and one, five pairs. Those submitting two or more pairs provided 75% of the cultures.

**Control specimens.** Two sets of controls were procured. Places, time, environmental conditions, and processing were the same as for the experimental group. The larger control group consisted of 45 specimens taken in groups of three specimens on random weekday mornings, averaging twice a month. For these the Todd-Hewitt Broth was poured directly from the vial into the kit, omitting actual mouth rinsing, labeled “mouthwash,” and assigned a fictitious name to ensure standard processing. A smaller set of controls consisted of six pairs of mouthwash-sputum specimens from two employees instead of patients.

**RESULTS**

**Controls.** No control specimen yielded acid-fast organisms.

**Experiments.** From 20 of the 113 patients (424 specimens), 37 mycobacterial cultures were obtained. Approximately one-third more isolations were made from those patients furnishing two or three pairs of specimens as compared to those patients providing only one pair of specimens each. The non-M. tuberculosis mycobacteria isolated from 19 patients constituted 87% of the total cultures isolated. Species in each of the four Runyon groups were isolated and M. intracellulare (although M. avium and M. intracellulare are indistinguishable by the procedures used, M. intracellulare is used throughout because no avian specimens were submitted) was isolated most frequently. Sputum specimens provided 59% of the non-M. tuberculosis cultures and the majority of these were M. intracellulare and M. kansasii (Table 1).

Examination of the data indicates that 25 of the 37 cultures were isolated from either mouthwash or sputum, but not from both members of the paired specimens, and only 1 of these cultures was M. tuberculosis.

In only three patients did both the mouthwash and sputum samples yield the same species of Mycobacterium. One patient provided three pairs of specimens from which M. phlei was isolated. One patient provided two pairs of specimens from which M. tuberculosis was isolated and one patient provided only one pair of specimens yielding M. smegmatis. Thus the same species of Mycobacterium, other than M. tuberculosis, was recovered in less than 2% of paired specimens. M. kansasii and M. intracellulare showed quite uneven distribution between mouthwash and sputum specimens; among 14 cultures from 12 patients, 4 were from mouthwashes
TABLE 1. Distribution of mycobacteria in 212 paired specimens of mouthwashes and sputum from 113 patients

<table>
<thead>
<tr>
<th>Specimens</th>
<th>M. tuberculosis</th>
<th>M. kansasii</th>
<th>M. scrofulaceum</th>
<th>M. intracellulare</th>
<th>M. terrae</th>
<th>M. phlei</th>
<th>M. smegmatis</th>
<th>Total MOTTa</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mouthwash only</td>
<td>1</td>
<td>1</td>
<td>3 (2)b</td>
<td>3 (3)</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>9 (8)</td>
</tr>
<tr>
<td>Sputum only</td>
<td>0</td>
<td>4 (2)</td>
<td>3 (3)</td>
<td>6 (6)</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>15 (12)</td>
</tr>
<tr>
<td>Mouthwash and sputum</td>
<td>4 (1)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>6 (1)</td>
<td>2 (1)</td>
<td>8 (2)</td>
<td></td>
</tr>
<tr>
<td>Totals</td>
<td>5 (2)</td>
<td>5 (3)</td>
<td>6 (5)</td>
<td>9 (9)</td>
<td>1</td>
<td>8 (3)</td>
<td>3 (1)</td>
<td>32 (19)</td>
</tr>
</tbody>
</table>

a Mycobacterium other than M. tuberculosis.
b Low catalase, nonpathogenic strain.
c Numbers in parentheses indicate the number of patients from whom the cultures were isolated.

and 10 were from sputa. On the other hand, four species (M. tuberculosis, M. phlei, M. scrofulaceum, and M. smegmatis) showed a more even distribution between mouthwashes and sputa; among 22 cultures from 10 patients, 12 cultures were isolated from mouthwashes and 10 were isolated from sputa.

Two species of Mycobacterium were isolated from the paired specimens from 4 of 113 patients as indicated in Table 2. Incorporated in Table 1, this accounts for seeming discrepancies in patient numbers. Two of these four patients also accounted for the M. tuberculosis and M. smegmatis recovery in both members of pairs.

From each of the two samples of tap water collected from a faucet at a nurses’ station, but not from the sample of water collected from a faucet in a patient’s room, M. intracellulare was isolated.

The rinsings from the fingers of 1 of 52 patients yielded M. intracellulare.

DISCUSSION

Failure to isolate mycobacteria from the control specimens indicates the mycobacteria were not introduced with the media and reagents or during the processing of the specimens, but came from the materials supplied by the patients.

Since most of the non-M. tuberculosis mycobacteria were rarely found in paired specimens, but often were found about equally distributed in mouthwashes and sputa, it is suggested that their presence is random and their acquisition may be by ingestion. The widespread finding of many of these organisms in soil, water (1, 4, 7), and pasteurized milk (2) supports a hypothesis that man acquires them by ingestion. From a survey of such mycobacteria in a New England hospital, Warring (8) concluded that the oropharynx constituted the major source of the microorganisms during passage through the mouth. However, the possibility should not be ignored that, following inhalation and ciliary transport from bronchi to the larynx, the organisms could be recovered from the mouth.

Two species of mycobacteria demonstrated a different pattern of residence in the host in that they were isolated more frequently from sputa. Inhalation would appear to be a more likely route of infection of the host. The greater incidence of M. kansasii in sputa, as compared to mouthwashes, is not accounted for by its being primarily a pulmonary pathogen, for M. tuberculosis was recovered at least as often in mouthwashes as in sputa. In the case of a PPD-S anergic patient, not included in this study, one mouthwash specimen was the only specimen from this patient from which M. tuberculosis was ever isolated in spite of several cultures of sputum specimens having been made. M. kansasii has been cited (4) for unusual distribution according to the age of the individual. The other organism which was more frequently isolated from sputa
than from mouthwashes was *M. intracellulare*, and, in spite of its prevalence, it was never isolated from both members of paired specimens. Its prevalence may be explained in part by the fact that it was the only mycobacterium to be isolated from tap water and from the fingers of patients during these studies.

A possible bearing upon the isolation of *M. intracellulare*, the most frequent of any Mycobacteria from the 165 patients in this study, was the observation that aides in at least three hospital locations handled the interiors of water and food containers being prepared for patients. This would support the hypothesis that ingestion was the route of transmission in some cases. As approximately one-third of the patients were outpatients, and others may have had specimens collected shortly after admission, it is reasonable to assume that many may have acquired their mycobacteria outside of the hospital. Waring (8) found that for the mycobacteria other than *M. tuberculosis*, the outpatients provided slightly more than the inpatients.

The random distribution of *M. intracellulare* between mouthwashes and sputa among the group of patients also suggests inhalation as a possible route of transmission. Following inhalation and ciliary transport from the bronchi towards the mouth, the organisms could then be recovered from the mouth. Environmental studies might assist in locating the sources of water-borne or airborne mycobacteria in the home or hospital for such devices as scrubbers and sprayers using water possibly containing the organisms in question produce aerosols. A 20-month survey of the cultures of clinical specimens showed that all mycobacterial species were fairly randomly distributed as to time and space. Such endemic occurrence simulates the data of Picken (9) which indicates a pattern characteristic of aerial infection in urban communities.

Random infection and recovery could be explained by normal body defense mechanisms keeping ordinary saprophytic or commensal organisms under control. However, Dubos (3) has pointed out that in some patients biochemical changes due to debilitating disease or therapy may so alter the immune response as to favor bacterial growth.

**LITERATURE CITED**